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U-101 - Rpt #4(Final)
Contract: DA19-129-qm-1836
University of Tennessee

A Study and Investigation of New Algae Strains

Period: 24 June 1961 - 23 September 1962

ARMED FORCES FOOD AND CONTAINER INSTITUTE
U. S. Army Research and Engineering Command
Chicago 9, Illinois

AD_____ Accession No._____

University of Tennessee, Knoxville, Tenn.
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W. R. Herndon
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CONTRACT RESEARCH PROJECT REPORT

ARMED FORCES FOOD AND CONTAINER INSTITUTE, CHICAGO
U. S. Army Quartermaster Research and Engineering Command
U. S. Army Quartermaster Research and Engineering Center,
Natick, Massachusetts

The University of Tennessee
Knoxville, Tennessee

Official Investigator:
Dr. Walter R. Herndon

Project Nr.: 7-84-13-002
Contract Nr.: DA19-129-qm-1836
Report Nr.: 4(Final)
File Nr.: U-101
Period: 24 June 1961 -
23 September 1962
Initiation Date: 24 June 1961

Title of Contract: A Study and Investigation of New Algae Strains

ISOLATION AND INVESTIGATION OF STRAINS OF ALGAE EXHIBITING OPTIMUM RATES OF GROWTH BETWEEN 25°C AND 60°C

Report 4
(Final Report)
Period 23 June 1961 - 23 Sept 1962

Summary of Previous Reports

Selective environmental conditions were created to screen for algae with desirable growth characteristics, specifically for those capable of rapid growth under high light intensity in media of defined composition. A modification of Bristol's solution (Report 1, Table 1) was used as the medium for initial screening; samples of soil and water from varied localities were inoculated in culture vessels and placed immediately adjacent to banks of fluorescent lamps; the temperature was adjusted to 35°C. Unialgal and bacteria-free cultures were isolated from mixed cultures which developed most rapidly.

From initial screening thirty-six strains capable of good growth under the conditions of selection were isolated. In addition forth-nine cultures from the Culture Collection of Algae of Indiana University were selected and screened for growth at 35°C in the same manner (Report 1, Table 2).

After consultation with the project officer, eighteen of the strains considered most promising were selected for comparitive evaluation with Chlorella 71105. Preliminary comparisons were made using a temperature of 38°C, Bristol's medium and two media offered by the project officer,

T-4 (Report 2, Table 1) and T-5 (Report 2, Table 2). Growth was estimated visually at first (Report 2, Table 4), subsequently, by direct cell counts (Report 2, Table 5) and turbidimetric readings (Report 2, Table 6).

A miniaturized rapid growth system was devised using a BOD incubator with normal operating range of 0-50°C, equipped with banks of fluorescent lamps providing approximately 800 ft candles intensity and with 5% CO_2 (in air) supply.* Growth (measured by packed cell volume and cells/mm^3) of the eighteen selected new strains and Ankistrodesmus falcatus were compared with Chlorella 71105 in 25 mm culture tubes, using 25 ml of T-4 medium, with continuous bubbling of CO_2 at 25°C and 35°C (Report 3, Table 1).

Work accomplished not included in Reports 1-3

The comparison of the eighteen selected strains, Ankistrodesmus and Chlorella 71105 was continued using the same conditions outlined above but at temperatures of 30°C and 40°C. Results of these studies, together with those reported in Report 3, Table 1, Experiment 1, are combined for ease of combined for ease of comparison in Table 1 of this report.

The isolates were tested for growth in darkness with glucose (20g/l) and proteose peptone (10 g/l) added to the inorganic (T-4) medium. The algal strains were placed in liquid medium (without added carbon dioxide) and on agar slants in light tight boxes and incubated for ten days at 35°C. The results are shown in Table 2. All but four of the isolates studied were capable of heterotrophic growth. The most conspicuously heavy growth occurred in strains NPlbT1, NPlcT1 and SPlaT3 at 35°C. Heterotrophic growth

*Insert A

*The details of the equipment set up including pertinent dimensions are shown in Figures 1 through 5.

was not studied at other temperatures.

On the assumption that, at some time, it may be desirable to consider the possibility of growing this type of organism in batches, without special apparatus at room temperature, uniform inocula were placed in 50 ml of T-4 medium in 250 ml erlenmeyer flasks and allowed to grow with atmospheric CO_2 for a period of 50 days. Results are shown in Table 3. Of the organisms tested, NPlbT3, OPlaTl, OplbTl, OPlcT3 and OpldT3 gave conspicuously high yields as measured by packed cell volume.

Additional morphological observations were made and a brief characterization of each of the strains is included as Table 4.

An academically oriented study, broadly within the scope of the contract but not directly a part of it, was made by Miss Patricia A. Manco who served as a research assistant for the contractor. The text of this study, submitted in partial fulfillment of the requirements of the M.S. degree, is summarized as follows:

A study of Two Lichen Phycobionts of the Genus Trebouxia in Culture

"Trebouxia decolorans and Trebouxia gelatinosa grew in light in defined inorganic media with glucose maintaining their typical morphology known from studies in a complete medium containing the less well defined additives of soil water and proteose-peptone. T. decolorans remained pigmented in all of the cultural conditions used including light intensities up to 300 ft.-c. T. gelatinosa lost its sheath in the absence of

glucose (but remained alive) in media with proteose-peptone present and the sheath failed to develop when certain concentrations of NH_4Cl or NH_4NO_3 were supplied as sources of nitrogen. Both species grew with nitrogen supplied as $NaNO_3$, NH_4Cl and NH_4NO_3. Growth was measured over a period of fifty days in culture supplied with six different concentrations of nitrogen supplied from each of the three sources. Maximum growth of T. decolorans occurred at a level of 300mg/liter $NaNO_3$ and maximum growth of T. gelatinosa occurred at a level of 136.8 mg/liter of NH_4NO_3. Growth judged to be most typical morphologically of both species occurred when nitrogen was supplied as $NaNO_3$, although the lag phase of cultures grown with nitrogen supplied as NH_4Cl and NH_4NO_3 was much shorter and in some cases the growth rate was higher. Nitrogen deficient cells were distinguishable by granulated cytoplasm, obscured chloroplasts, yellowing o loss of color and increase in metabolities, including lipid content. Morphological variation was more pronounced in cultures supplied with NH_4Cl or NH_4NO_3 than with $NaNO_3$, there being marked variation in shape and sharply increased fat content, these perhaps being associated with pH changes. In a study of the uptake of $C^{14}O_2$ it was found that three to six times more carbon was fixed in the absence of glucose in light than in the presence of glucose in light, although glucose is required for the continued growth of both organisms in the basal inorganic medium."

Discussion

Since the ultimate objectives of the project are related to yield of plant substance rather than to rate of cell division, the determinations of growth by packed cell volume are probably of more significance than determinations made by other methods. From this standpoint it appears that, under certain conditions, a number of the new isolates might prove superior to Chlorella 71105 in a rapid growth system.

Strains of algae isolated which equaled or exceeded yield (measured by packed cell volume) of Chlorella 71105 in T-4 medium (both being grown at the temperature indicated) were,

at 40°C	at 35°C
CPlaT1	BTlaT1
CPlbT3	NPlbT1
NPlbT1	NPlbT3
NPlbT3	NPlcT1
NPlcT1	
SPlbT2	
at 30°C	**at 25°C**
NPlbT3	All other isolates studied
NPlcTI	
OPlaT1	

Strains of algae which equaled or exceeded the maximum yield (packed cell volume) obtained (at 35°C) for Chlorella 71105 under any of the conditions used were:

BTlaT1 (at 35°C)
NPlbT3 (at 25° and 35°C)
NPlcT1 (at 30°C)
OPlaT1 (at 30°C)

Of the strains studied, several grew about equally well over a wide temperature range: These included the Ankistrodesmus strain, AR2DT1, AR2ET1, ChlaTO and CHlbTO (between 25 and 35°C), and CPlaT1, CPlbT3 and SPlaT3 (between 25 and 40°C). The others displayed more growth at one temperature used, as follows: BTlaT1 (35°C), BTlbT1 (35°C), Chlorella 71105 (35°C), NPlbT1 (35°C), NPlbT3 (35°C), NPlcT1 (30°C), OPlaT1 (30°C), OPlbT1 (35°C), OPlcT3 (30°C), OPldT3 (30°C), SPlaT2 (35°C) and SPlbT2 (25°C).

It is apparent that there are a large number of green algae capable of growth about 25°C but that relatively few grow well above 40°C. It is also clear that each of the strains submitted will have their own particular advantages and disadvantages for potential use in rapid culture devices and as unconventional foods. If they are to be used in a particular kind of culture device, such as that used by the project officer, it seems to me that the quickest and easiest way to further evaluate the potential use of the strains isolated, would be to make inoculations of a number of the strains into the system simultaneously. The strain or strains best suited to the system should outgrow the others and could be quickly reisolated. Since in any growth system there are apt to be at least some fluctuations, it may be desirable to consider the possibility of running several strains of algae in the system at the same time regularly. Strains selected to take advantage of any potential fluctuations could contribute to stability of yeild although, unless carefully selected, it would introduce a potential

problem of variation in the nature of the yield.

The use of cultures at or near temperatures of 37°C has one important potential hazard. The probability that bacterial contmainants in the system might be pathogenic is increased. While it is easy enough to maintain bacteria-free cultures in the miniaturized system reported here, it is considerably harder on a mass scale. In mass culture, it may be desirable to accept a lower yield and work at a lower or higher temperature to reduce this hazard.

One set of the cultures isolated and used in this study has been delivered, in person, to the project officer. A second set is being shipped with this report. Two other sets are extant and will be kept in the laboratories of the University of Tennessee for a period of six months (or as long as it is practicable and of value to the government).

Additional duplicates or opinions on maintenance and manipulation of the cultures will be submitted if needed.

Walter Herndon
University of Tennessee
Knoxville, Tennessee

TABLE 1

Growth of algal strains in miniaturized rapid growth system:

Light intensity - 800 ft-candles

Organism	25°C		30°C		35°C		40°C	
	Packed cell vol. 25 ml	Cells/ c.mm.	Packed cell vol.	Cells/ c.mm.	Packed cell vol.	Cells/ c.mm.	Packed cell vol.	Cells/ c.mm.
Ankistrodesmus	.02	15	.02	57	.02	13	-	-
AR2-DT.1	.02	20	.01	53	.02	68	-	-
AR2-ET.1	.02	50	.02	150	.02	79	-	-
BT1-aT.1	.05	95	.03	52	0.18	700	-	-
BT1-bT.1	.02	15	.03	14	.05	42	-	-
CH1-aT.0	.04	25	.04	36	.03	16	-	-
CH1-bT.0	.03	15	.03	23	.02	11	-	-
CP1-aT.1	.02	45	.03	100	.03	175	.03	54
CP1-bT.3	.03	20	.03	50	.03	27	.03	14
Chlorella 71105	.02	35	.07	700	.08	1750	.02	85
NP1-bT.1	.07	150	.06	29	0.1	144	.07	17
NP1-bT.3	.08	100	.07	180	0.15	544	.03	13
NP1-cT.1	.07	25	.09	220	0.1	146	.04	19
OP1-aT.1	.02	45	.08	550	.05	39	-	-
OP1-bT.1	.03	25	.05	125	.06	750	-	-
OP1cT.3	.02	20	.05	150	.04	675	-	-
OP1-dT.3	.03	30	.05	140	.04	94	-	-
SP1-aT.2	.02	18	.04	26	.05	35	-	-
SP1-aT.3	.03	20	.02	38	.03	232	.02	40
SP1-bT.2	.07	25	.03	29	.03	61	-	-

TABLE 2

Packed cell vol. of test organisms cultured in 50 ml. of T-4 Medium at 25°C for 50 days - atmospheric CO_2.

Test Organism	Packed-cell Vol.	Test Organism	Packed-cell Vol.
Ankistrodesmus	0.04	NPl-bT.1	0.25
AR2-DT.1	Not Tested	NPl-bT.3	0.30
AR2-ET.1	Not Tested	NPl-cT.1	Not Tested
BTl-aT.1	0.20	OPl-aT.1	0.42
BTl-bT.1	0.17	OPl-bT.1	0.38
CHl-aT.0	Not Tested	OPl-cT.3	0.35
CHl-bT.0	0.04	OPl-dT.3	0.34
CPl-aT.1	0.06	SPl-aT.2	0.04
CPl-bT.3	0.07	SPl-aT.3	0.04
Chlorella 71105	0.16	SPl-bT.2	0.07

TABLE 3

Ratings of test organisms cultured at 35°C in darkness on both agar and liquid medium, growth evaluated subjectively after 10 days.

Organism	Agar Slants	Liquid Medium
Ankistrodesmus	+	+
AR2+DT.1	+	0
AR2-ET.1	+	+
BT1-aT.1	+	+
BT1-bT.1	+	+
CH1-aT.0	+	++
CH1-bT.0	0	0
CP1-aT.1	++	0
CP 1-bT.3	+	+
Chlorella 71105	0	0
NP1-bT.1	++	+++
NP1-bT.3	0	0
NP1-cT.1	+++	+++
OP1-aT.1	+	+
OP1-bT.1	+	+
OP1-cT.3	0	0
OP1-dT.3	+	+
SP1-aT.2	+	+
SP1-aT.3	+++	+++
SP1-bT.2	+	+

0= no growth
+= poor
++= fair
+++= good

TABLE 4

GENERAL MORPHOLOGICAL FEATURES OF STRAINS STUDIED

Culture No.	Generic Determination	Motility	Cell shape	Av. Cell Size	Chloroplast	Pyrenoid	Starch Test	Fat Test
IU-101	Ankistrodesmus	0	Acicular	3.5x40 u	Parietal	-	+	-
AR2-Dt1	Chlorococcum	Walled Zoospores	Ellipsoid to spherical	10.4 u	Parietal	Parietal	+	-
AR2-eT1	Chlorococcum	Walled Zoospores	Ellipsoid to spherical	8.5 u	Parietal	1-2 Peripheral	+	+ -
BT1-aT1	Chlamydomonas	+	Ellipsoid	6.5x10.4	Cup-like	Posterior	+	-
BT1-bT1	Chlamydomonas	+	Spherical or nearly so	8.5 u	Cup-like	Posterior	+	-
CH1-aT0	Chlamydomonas	+	Ellipsoid	8.5x13 u	Parietal to Cup-like	Parietal or Posterior	+	-
CH1-bT0	Chlamydomonas	+	Spherical to Ellipsoid	10.4x 13.2 u	Cup-like	Posterior	+	+
CP1-aT1	Ankistrodesmus	0	Sickle-like	3.9x14.3	Parietal	-	-	-
CP1-bT3	Neochloris	Naked Zoospores	Spherical	13. u	Parietal	Parietal	+	-
71105	Chlorella	0	Spherical	4.8 u	Parietal	-	+	-
NP1-bT1	Chlorococcum	Walled Zoospores	Ellipsoid to Spherical	14 u	Parietal	Parietal	+	+
NP1-bT3	Chlorococcum	Walled Zoospores	Ellipsoid to Spherical	16.2u	Parietal	1 to many Parietal	+	+ -
NP1-cT1	Chlamydomonas	+	Spherical or nearly so	15.6	Cup-like	Central or Posterior	+	- +
OP1-aT1	Chlamydomonas	+	Ellipsoid or tapered post.	8.4x11 u	Cup-like	Usually central	+	-
OP1-bT1	Chlamydomonas	+	Ellipsoid with broad wall	6.5x13 u	Cup-like	Posterior	+	+
OP1-cT3	Chlamydomonas	+	Ellipsoid	4.5x10.4	Cup-like	Central to Posterior	+	+
OP1-dT3	Chlamydomonas	+	Ellipsoid to Spherical	9.8	Cup-like	Posterior	+	-

TABLE 4 continued

Culture No.	Generic Determination	Motility	Cell shape	Av. Cell Size	Chloroplast	Pyrenoid	Starch Test	Fat Test
SP1-aT2	Chlamydomonas	+	Ellipsoid	9.4x11.7 u	Parietal or Cup-like	Parietal or Posterior	+	+
SP1-aT3	Chlorella	0	Spherical	4.9u	Parietal	-	+ -	-
SP1-bT2	Chlamydomonas	+	Ellipsoid	8.5x12.4	Cup-like	Central to Posterior	+	+

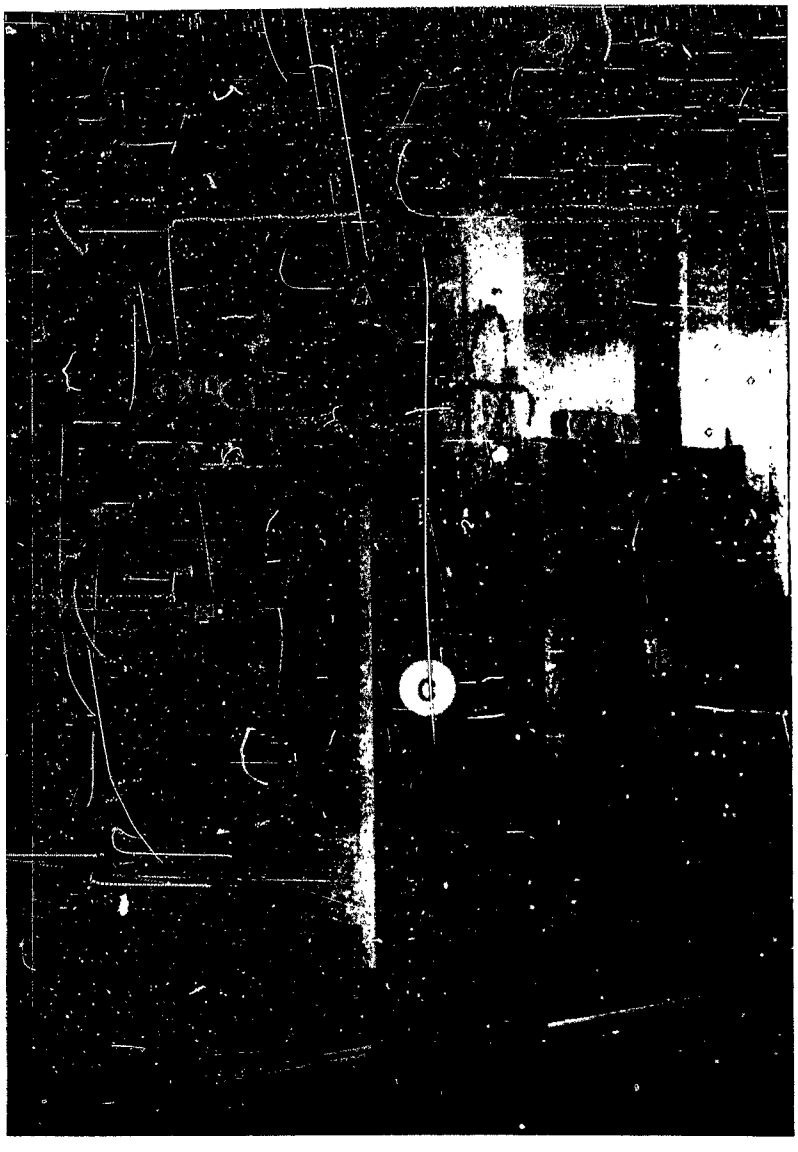

Figure 1: General view of growth chambers: Basic units are Precision BOD incubators (a) (b) model 805 (Catalog no 31213). Gas supply (c) is 5% CO_2 in air.

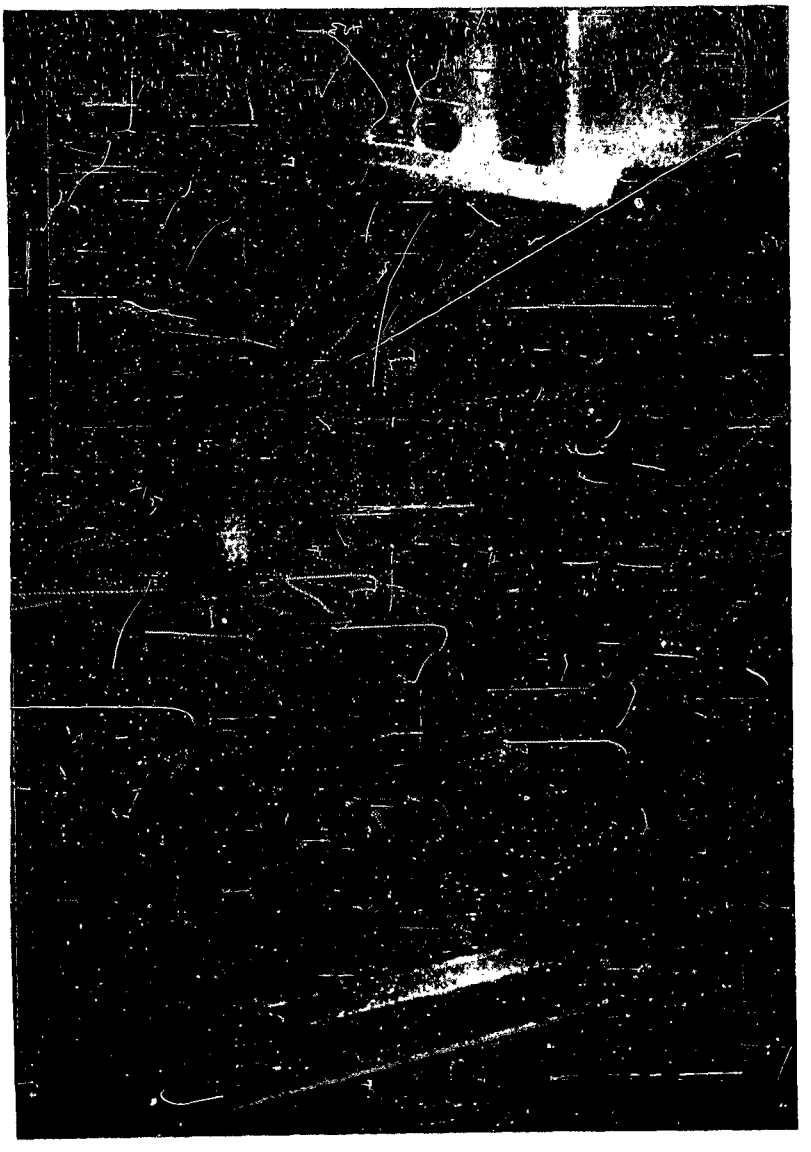

Figure 2: Open growth chamber showing: (a) Temperature control system with (left to right) thermostat, pilot lamp, switch and temperature dial, (b) Heating-cooling compartment (c) Illuminated chamber, (d) Ballasts for fluorescent lamps mounted externally.

CO_2 passes through water trap (on top of cabinet) and through opening above ballast wiring.

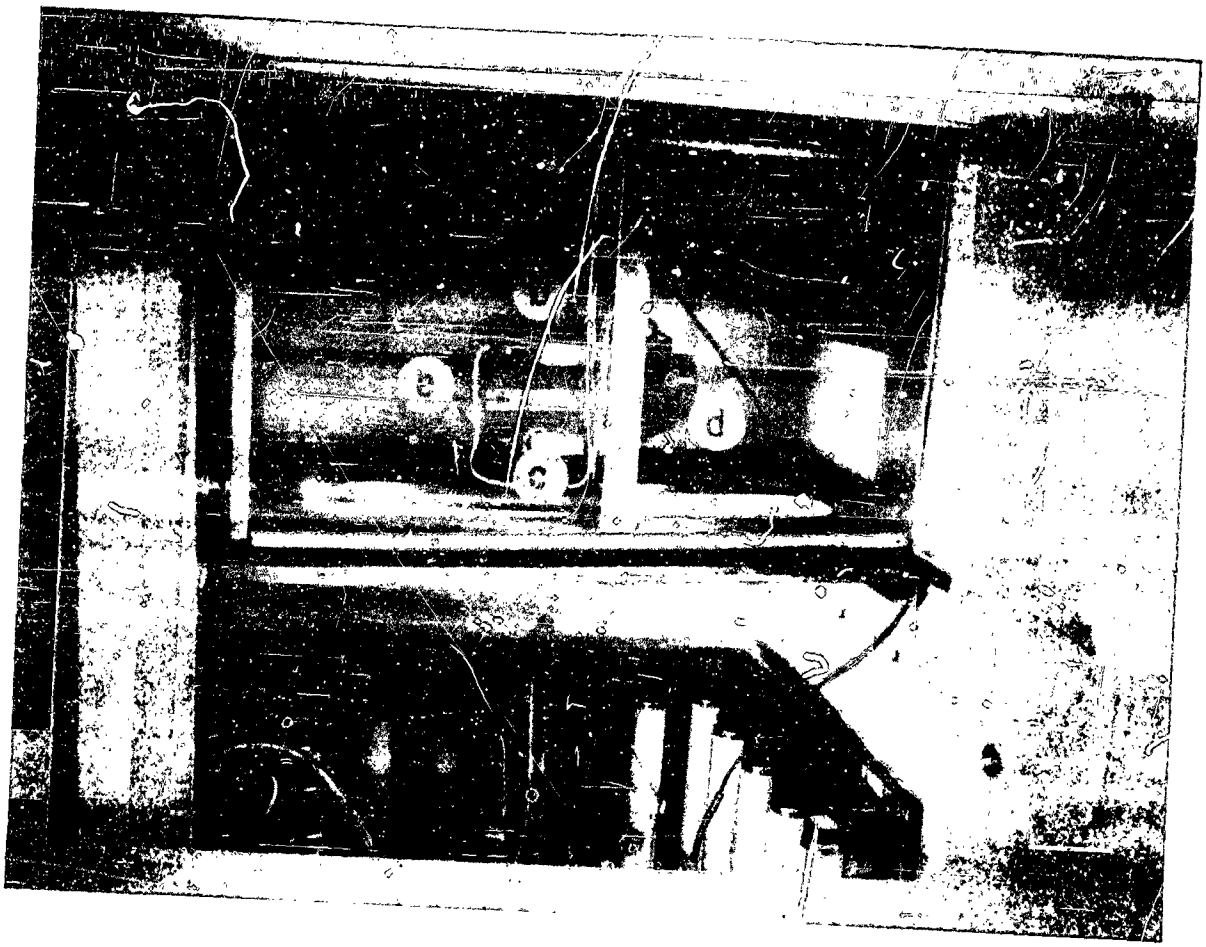

Figure 3: Detail of Heating-cooling compartment. Refrigeration compartment of the Precision BOD incubator is a standard General Electric model (a) with two heaters (b) (c) (150 and 250 ohms), a fan (d), and a dual position hydraulic thermostat (e) added.

Below may be seen CO_2 inlet to manifold in illuminated chamber. Manifold is 1 1/2" in diameter with individually controlled needle valves outlets

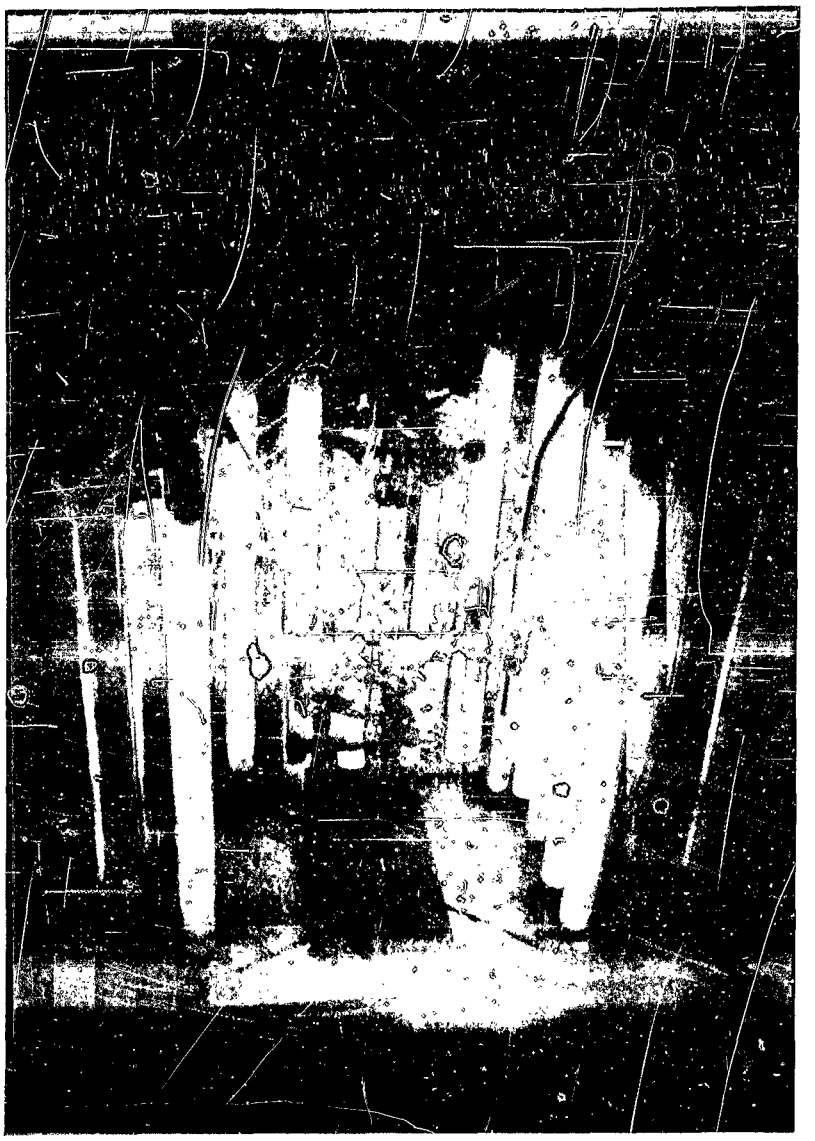

Figure 4: Detail of Illuminated Chamber: Fifteen fluorescent (23 inch cool white) tubes placed in three rows 8 cm apart (measured between centers). Left row and right row are 38 cm apart; back row of tubes is 40 cm from reflective surface of door. Culture tubes and manifold are mounted on ring stand with adjustable clamps. Temperature is measured by thermometer in culture fluid (center tube). Culture tubes (25 x 200 mm) are positioned, by photometer, individually to receive uniform illumination; average distance from center of culture tube to nearest lamp is 9.5 cm. CO_2 passes into cultures through glass pipettes with tips placed close to the bottom of the tubes so that passage of gas constantly stirs the cultures.

Figure 5: Facilities for maintaining stock cultures.